

- A. INFLUENCE OF VARIOUS CARBOHYDRATES
ON THE UTILIZATION OF PROTEIN BY
THE ALBINO RAT.
- B. INADEQUACY OF UNMODIFIED POTATO
STARCH AS A DIETARY CARBOHYDRATE
FOR THE ALBINO RAT.

by

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ABSTRACT

The influence of various carbohydrates on the utilization of dietary protein by the albino rat was investigated. The methods of evaluating the diets consisted of growth experiments, protein digestibility studies, and the determination of certain of the essential amino acids in the feces.

Potato starch, autoclaved potato starch or dextrin each induced greater levels of amino acids in the feces of rats than either sucrose or glucose. The inclusion of 10% of lactose in the sucrose ration resulted in an increase in the levels of lysine and histidine in the feces. The amino acids found in feces appeared to be derived chiefly from endogenous sources.

Rats fed ad libitum a ration containing 9% casein and 0.5% methionine with corn starch as the source of carbohydrate, grew at a higher rate than those fed a corresponding ration with sucrose as the source of carbohydrate. The nutritional efficiency of the sucrose ration was improved when histidine, valine, threonine, lysine, and tryptophan were included in small quantities.

Unmodified potato starch as the source of carbohydrate in the diet of the rat was poorly utilized and also induced a decrease in the digestibility of dietary protein. The poor utilization of unmodified potato starch was attributed to the resistance of the outermost layers of the starch granules to attack by alpha-amylase.

THE UNIVERSITY OF ALBERTA

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SECTION A

INFLUENCE OF VARIOUS CARBOHYDRATES ON THE UTILIZATION OF PROTEIN BY THE ALBINO RAT

INTRODUCTION

The protein sparing action of dietary carbohydrates has been extensively studied and has recently been comprehensively reviewed by Munro (31). However, little attention has been paid to the influence of different carbohydrates on the utilization of low protein diets. There is some evidence to indicate that protein utilization may be modified by altering the carbohydrate fed. Harper and Lepp (9) observed that only when the level of casein was increased from 18% to 20% or 22% did chicks receiving sucrose diets grow as well as those fed dextrin as a source of carbohydrate.

Hall and Sydenstricker (8) found that rats, receiving a 9% casein diet containing added methionine and with sucrose as the carbohydrate, grew at about half the normal rate. Normal growth was obtained when histidine, lysine, valine, threonine, and tryptophan were added to the diet. However, normal growth was also obtained when sucrose was replaced by corn starch, and inositol, p-aminobenzoic acid, and nicotinic acid were added. Hall and Sydenstricker suggested that with the 9% casein diet, corn starch and additional vitamins promoted intestinal synthesis which made up the small deficiencies in essential amino acids other than methionine.

Krehl, Henderson, de la Huerger, and Elvehjem (20) noted

that relatively good growth of rats was obtained when dextrin or corn starch was employed as the carbohydrate in 9% casein diets low in nicotinic acid. The addition of nicotinic acid or tryptophan did not benefit growth significantly. However, when sucrose was used as the carbohydrate, growth was poor. The addition of nicotinic acid gave only a partial improvement, whereas the addition of tryptophan resulted in still better growth.

The present investigation was designed to study the influence of various carbohydrates on the utilization of low protein rations by the albino rat. The methods of evaluating the diets consisted of growth experiments, protein digestibility studies, and the determination of certain of the essential amino acids in the feces.

REVIEW OF LITERATURE

In 1909, Kendall (19) demonstrated that the nature of the diet determined the dominant types of intestinal bacteria. Using monkeys and cats, he found that a carbohydrate diet produced an acidophilic type of fecal flora, whereas proteolytic bacteria predominated when the animals were placed upon a purely protein regimen. Similarly, Hull and Rettger (17) showed that the character of the intestinal flora of the rat could be altered by changing the diet from the ordinary mixed food to a synthetic diet containing milk or lactose. Such a change quickly resulted in a marked simplification of the intestinal flora. Bacillus coli and other intestinal organisms were practically eliminated, while bacilli of the type of Bacillus acidophilus predominated. Later, Porter and Rettger (34) studied the influence of diet on the distribution of bacteria along the alimentary tract of the rat. On a stock diet of calf meal, the "normal" bacterial flora of the young white rat was found to be comparatively simple, especially in the upper segments of the alimentary tract, the flora being made up largely of the Lactobacillus acidophilus type of aciduric organisms. Bacteria became increasingly more abundant in the lower sections of the small intestine, the lower ileum and caecum. These regions contained appreciable numbers of coli-like organisms. The "normal" bacterial flora throughout the digestive tract

could be altered by certain foods or by starvation. Gall, Fenton, and Cowgill (6) have shown that the intestinal flora of mice fed a dextrose diet is different from that of mice maintained on a ration containing dextrin.

The possibility of the intestinal bacteria making an essential contribution to the nutrition of the host was suggested when several investigators found that coprophagy reduced the B-vitamin requirements of the rat. Steenbock, Sell, and Nelson (43) found that rats kept on screens required 2 to 3 times as much yeast in the ration as a source of B-vitamins as animals kept on shavings. Heller, McElroy, and Garlock (13) were able to show that spore-bearing organisms, found in the feces of rats, synthesized B-vitamins. Roscoe (36) confirmed earlier investigations showing that coprophagy prolonged the life of rats receiving diets deficient in the vitamin B-complex. She suggested that coprophagous rats were able to utilize the B-vitamins in the feces that had been synthesized by bacteria in the caecum.

In 1935, Guerrant, Dutcher, and Tomey (7) found that the type of carbohydrate used in the diet influenced the amount of B-vitamins found in the feces. Dextrinized corn starch was more effective than sucrose, glucose or commercial corn starch in promoting the synthesis of B-vitamins. Lactose appeared to occupy an intermediate position between dextrin and sucrose in this respect. On finding that B-vitamin forma-

tion occurred in the caecum of the rat, Guerrant et al. suggested that the peculiar property of dextrinized corn starch resulted from the incomplete digestion of this particular form of carbohydrate; the undigested carbohydrate, on reaching the lower part of the digestive tract of the rat, presumably stimulated the growth of the intestinal microorganisms which were believed to be the specific agents synthesizing the B-vitamins. This view was later supported by Schweigert, McIntire, Henderson, and Elvehjem (41) who showed that caecectomy or the feeding of sulfasuxidine decreased the synthesis of riboflavin in the intestinal tract of rats which received either lactose or dextrin as the only carbohydrate. With sucrose diets, caecectomy or sulfasuxidine administration caused little effect on the riboflavin level in the intestinal tract. The importance of the amount of carbohydrate reaching the caecum of the rat in promoting the intestinal synthesis of the B-vitamins was also shown by refection, whereby spontaneous synthesis of B-vitamins occurs in the intestinal tract of rats fed either raw potato starch or raw rice starch as the source of carbohydrate. (1,18,36).

The conversion of non-protein nitrogen to protein by the microorganisms of the rumen and the subsequent digestion and utilization of this protein by the ruminant has received much attention. The more important investigations between 1937 and 1947 have been extensively reviewed by McNaught and Smith (25).

from the results of their feeding experiments, Hart, Bohstedt, Deobald, and Wegner (11) concluded that urea and ammonium bicarbonate nitrogen could be used by growing calves to replace a portion of the dietary protein. Later, a procedure was used by Mills, Booth, Bohstedt, and Hart (26) whereby rations with and without urea were given to heifers through a rumen fistula. The ammonia and crude protein in the rumen contents were estimated at intervals after feeding. A decrease in ammonia and an increase in crude protein after the ingestion of urea were regarded as evidence of the conversion of ammonia nitrogen to protein. Mills et al. found that the utilization of N.P.N.^{*} occurred to a significant extent only when the ration contained ample starch and was low in protein. That ammonia was converted to protein in significant amounts only when low protein rations were fed had also been observed by Wegner, Booth, Bohstedt, and Hart (46). They found that with urea added to a basal ration containing 8.5% of protein no clear indication of protein synthesis was obtained. When the basal ration contained 4% protein, the addition of urea resulted in an increase in the crude protein content of the rumen ingesta. Harris and Mitchell (10), using nitrogen balance studies, showed that the addition of urea to a low protein ration converted the ration into one that was capable of promoting a nearly normal rate of growth of lambs. They also showed that such a ration need contain no more than 11% of conventional protein, when urea provided 50% of the total nitrogen. Similarly, Hart et al.

* non-protein nitrogen

had shown that when urea supplied 43% of the total nitrogen, the ration gave far better utilization than when urea supplied 61% and 70% of the total nitrogen.

Pearson and Smith (33) devised a technique to study rumen synthesis in vitro. The rumen contents were strained through muslin to remove coarse particles and the resulting liquid was incubated at 39 °C for 3 to 4 hours under conditions similar to those in the rumen. Within the first 3 hours, the total N.P.N. diminished while the total nitrogen remained unaltered. The results supported the view that synthesis of protein occurred. That this synthesis was microbiological in origin was shown by the decrease in synthesis which occurred when the rumen fluid was incubated with minute amounts of toxic substances. Further investigations by Pearson and Smith showed that protein synthesis from ammonia, and protein breakdown with the production of ammonia, appeared to proceed at the same time in rumen contents. However, synthesis exceeded breakdown when starch, maltose or galactose was present. The stimulating effect of carbohydrates on protein synthesis in vitro was confirmed by Smith and Baker (42). They found that hydrolysis of protein predominated in the absence of carbohydrate, but when either glucose or maltose was added, marked synthesis was observed. The greater portion of the synthesis was attributed to the masses of microiodophilic bacteria which abound in the rumen. Protozoa did not appear to contribute to the synthesis.

Recently, attention has focused on the synthesis of individual amino acids in the rumen. Thomas, Loosli, Ferris, Williams, and Maynard (44) compared purified diets, one containing casein and the other an equal nitrogen level of urea as the only source of nitrogen. Growth of goats and sheep was poorer on the urea diet than on the casein diet, but the essential amino acid content of the rumen materials was similar for both groups of animals. Block and Stekol (2) fed sodium sulfate containing radioactive sulfur to a cow and collected milk from the animal for several days. The proteins were isolated, hydrolyzed, and the amino acids separated on chromatograms. Both cystine and methionine were found to contain radioactive sulfur in appreciable amounts. Thomas, Loosli, Williams, and Maynard (45) noted that growing lambs fed a purified diet low in sulfur exhibited gradual failure of appetite, loss of body weight and death. In the absence of dietary sulfur, urea nitrogen was apparently not utilized, since deficient lambs were consistently in negative nitrogen and sulfur balance. Earlier investigations by Loosli and Harris (22) had shown that when the basal diet was supplemented with urea and methionine, nitrogen retention by lambs was significantly greater than when the ration was supplemented with urea alone or with urea and sodium sulfate. Williams and Moir (47) confirmed these results. They suggested that the value of methionine as a supplement to urea in improving nitrogen retention in growing lambs was due largely to its stimulating effect on

bacterial growth in the rumen, which increased the amount of bacterial protein available to the host.

McNaught (24) has shown by experiments in vitro that the lysine content of the rumen liquid increases after incubation. This increase was estimated to represent a 12% increase in the lysine of whole rumen contents.

Current evidence supports the view that not only can N.P.N. indirectly support nitrogen metabolism in ruminants, but that also in the rat, the nitrogen of ammonium salts and certain other compounds can replace amino acid nitrogen in metabolism and growth. Rittenberg, Schoenheimer and Keston (35) fed rats a purified diet containing 15% casein and ammonium citrate which contained isotopic nitrogen. The proteins from the liver, intestine, kidney, and muscle of these rats contained significant amounts of isotope. Labelled nitrogen appeared in the glutamic acid and glycine prepared from liver and intestinal protein, in the arginine from all the proteins, and in the aspartic acid from liver protein. It was claimed that the foregoing results demonstrated the ability of the rat to utilize ammonium nitrogen for amino acid formation. Rose, Smith, Womack, and Shane (37) found that young rats grew slowly when restricted to a ration in which the nitrogen was provided by the essential amino acids alone. When the non-essential amino acids were added, good growth occurred. When the non-essential amino acids were replaced by diammonium citrate or L-glutamic acid containing an equivalent

amount of nitrogen, excellent growth was observed. Neither glycine nor urea was as effective as diammonium citrate or L-glutamic acid in providing non-protein nitrogen for growth. Lardy and Feldott (21) conducted experiments in which rats were given a ration producing a minimal positive nitrogen balance; when diammonium citrate was added, a marked retention of nitrogen occurred. No added growth was produced by adding sodium citrate, showing that it was the ammonium nitrogen which was active. The results obtained by Frost and Sandy (5) confirmed those of other investigators. They suggested that the best utilization of amino acid mixtures occurred when at least 20% of the total nitrogen was present as nitrogen other than that of the essential amino acids; this 20% of nitrogen appeared to be fairly non-specific.

The possibility that the intestinal microorganisms of the rat play a role in the synthesis of the non-essential amino acids was suggested by Martin (23). He found that the inclusion of sulfasuxidine in the diet caused a loss of weight in rats fed the 10 essential amino acids as a source of nitrogen. In attempting to explain his results, Martin proposed that certain amino acids were synthesized in the intestinal tract and thus did not appear essential. He concluded that the inclusion of sulfasuxidine reduced this synthesis and caused the deficiencies to be precipitated. William and Watson (48) fed arginine-free diets with and without sulfasuxidine to rats. Since there was no dramatic weight loss by the rats fed the

arginine-free diet containing sulfasuxidine, these investigators concluded that the intestinal tract was not the source of the arginine synthesized by the rat. Rose and Smith (38) further investigated the role of alimentary microorganisms in the synthesis of non-essential amino acids and obtained inconclusive results. With a ration containing the 10 essential amino acids in moderate excess, the presence of sulfasuxidine led to a slight, but statistically significant decrease in the rate of gain. However, the drug was without effect upon the growth of the rats which consumed a ration containing diammonium citrate and the 10 essential amino acids at their tentative minimum level. Rose and Smith suggested that the microorganisms which are inhibited by sulfasuxidine may play a role in the synthesis of non-essential amino acids, but that this role is of minor importance. They concluded that either microorganisms which are not affected by sulfasuxidine, or the cells of the host must be largely responsible for the synthesis of non-essential amino acids.

PART 1

EFFECT OF VARIOUS CARBOHYDRATES
ON THE LEVEL OF AMINO ACIDS IN THE FECES

Experimental

Animal-feeding techniques

Weanling male rats of 40 to 50 gram body weight (21 to 25 days) were used as the experimental animals. Each animal was housed in an individual cage having an elevated bottom of one-half inch mesh screen. The animals were weighed weekly and individual food consumption records were kept.

Groups, consisting of 5 to 6 animals each, were fed the experimental rations for a period of either 4 or 6 weeks. The food consumption of rats fed diets containing 18% casein was controlled by restricting the amount of ration offered. Equal quantities of food were offered during the first few days, but when the average food intake reached 10 g. per day, the ration thereafter offered was restricted to this amount. Ad libitum feeding was employed with rats fed diets containing 9% casein. Water was supplied ad libitum to all rats.

The composition of the basal rations is shown in Table I and the vitamin supplements used are shown in Table II. The levels of vitamins and minerals used were estimated to be optimal for the rat.

The various experimental rations are listed in Table III. The amounts of supplements added to the rations are reported

TABLE I

Percentage Composition of Basal Rations

casein	18	9
salt mixture*	4	4
corn oil	4	4
inositol	0.1	0.1
choline chloride	0.2	0.2
whole liver powder**	0.1	0.1
carbohydrate	73.6	82.6
	100.0	100.0

*salt mixture 351 (16) with the following changes: NaF was omitted; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ was increased from 0.35 g. to 16.2 g.; 60 g. of FeSO_4 was substituted for 20.5 g. of $\text{FePO}_4 \cdot 4\text{H}_2\text{O}$; 800 g. Na_2HPO_4 and 0.52 g. of ZnCl_2 were added.

**Wilson's whole liver powder.

TABLE II

Vitamin Supplements

	added to each kilo. of ration
thiamine hydrochloride	5 mg.
riboflavin	10 mg.
pyridoxine hydrochloride	5 mg.
nicotinic acid	5 mg.
calcium pantothenate	25 mg.
para-aminobenzoic acid	300 mg.
alpha-tocopherol	25 mg.
menadione	2 mg.
folic acid	2 mg.
biotin	200 mcg.
haliver oil	60,000 I.U. vitamin A & 12,000 I.U. vitamin D

TABLE III
Experimental Rations

<u>Exp. No.</u>	<u>% Casein</u>	<u>Ration No.</u>	<u>Ration</u>
I	18	1	potato starch
		2	sucrose
		3	autoclaved potato starch ★
		4	glucose
		5	dextrin ★★
II	18	6	potato starch
		7	potato starch & 20% lactose
		8	sucrose
		9	sucrose & 10% lactose
III	9	10	potato starch & 42.6% sucrose
		11	potato starch & 42.6% sucrose and 0.619% N
		12	autoclaved corn starch ★
		13	autoclaved corn starch & 0.619% N
		14	corn starch
		15	corn starch & 0.619% N

★ autoclaved starches were prepared by moistening the starch with water and autoclaving at 120°C for 2 hours. The product was dried in the air at room temperature and then ground in a Wiley mill.

★★ dextrin was prepared by baking thin layers of potato starch in an oven at 145°C for 8 hours with frequent stirring. The product gave a purple coloration on addition of iodine.

as percentages of the diets and were added at the expense of the carbohydrate. With the exception of sucrose, all the carbohydrates used in these experiments have been reported as favoring the synthesis of B-vitamins by the intestinal flora. During the first week of experiment II, it was found that 20% lactose incorporated in the sucrose diet caused diarrhea; therefore, the level of lactose was decreased to 10%. In experiment III, either 5.00% of ammonium citrate or 3.49% of ammonium bicarbonate was incorporated into rations 11, 13, and 15. The levels of ammonium salts used provided 0.619% nitrogen.

It was previously noted that rats could not be satisfactorily maintained on a 9% casein diet with potato starch as the only source of carbohydrate. Therefore, in rations 10 and 11 of experiment III, 40% of potato starch was used and the remainder of the carbohydrate consisted of sucrose. Coates, Henry, Kon, Kon, Mawson, Stanier and Thompson (4) found that refecation was obtained when potato starch made up 40% of the ration.

Analyses of feces for amino acids

Feces excreted during the first three days of the experiment were discarded. Thereafter, the feces from each rat were collected at least twice weekly from absorbent paper that had been placed beneath the cage. The feces were screened from food particles and hair, and were then

air-dried at room temperature, weighed, and ground for the analyses.

The proteins in one gram samples of the feces were hydrolyzed by autoclaving in 20 ml. of 5 N HCl for 10 hours at 120°C. The amino acids were determined by means of microbiological assays using essentially the same procedure as Henderson and Snell (15). Leuconostoc mesenteroides P. 60 was used as the test organism for lysine determinations, Lactobacillus arabinosus for valine, and Streptococcus fecalis for methionine and histidine. A Cannon titrator (14) with a quinhydrone electrode was used throughout for titrations.

Protein digestibility studies

The animals were housed in cages with one-quarter inch wire screen bottoms. The cages were supported by metal funnels. To collect the feces, a fine mesh screen was placed a few inches above the inverted apex of each funnel. The feces were removed every second day and were air-dried for twenty-four hours before being analyzed for nitrogen by the micro-Kjeldahl method (3).

The techniques followed were similar to those used by Mitchell (27). For the determination of metabolic nitrogen loss, each group, consisting of three young male rats, was placed on a ration containing 4% egg albumen. Each group was then fed a test ration containing 9% casein. In changing

from one ration to another, at least three days were allowed to elapse before the feces were again collected and analyzed. The carbohydrates tested for their effect on protein digestibility were corn starch, potato starch, autoclaved potato starch, and sucrose. The basal composition of the 9% casein ration used is shown in Table I. The low-nitrogen diet had the same general composition except that 4% egg albumen was substituted for 9% casein; the difference was made up by increasing the amount of carbohydrate.

Results and Discussion

Groups of rats fed rations containing sucrose, glucose, dextrin or autoclaved potato starch as the source of carbohydrate exhibited no marked growth differences under the conditions of experiment I. However, when unmodified potato starch was used as the only source of carbohydrate in the diet, the growth rate of the rats was low. The rats on this ration excreted white, bulky feces which were found by microscopic examination to consist largely of undigested starch granules. Also, the digestibility of dietary protein was shown to be impaired when unmodified potato starch was used as the sole source of carbohydrate (Table IX). Apparently the lower rate of growth of rats fed the unmodified potato starch ration was due to the poor utilization of unmodified potato starch and to the coincident decrease in the digestibility of the dietary protein.

The incorporation of lactose at a level of 20% into the unmodified potato starch ration improved the growth rate slightly, whereas the incorporation of 10% of lactose into the sucrose ration did not cause any observable change in the growth of rats. Mitchell (29), using diets containing various levels of lactose combined with corn starch, found that the growth of rats was practically normal on diets containing 30% or less of lactose. Similarly, Outhouse, Smith, Merritt, and White (32) observed that there was no

TABLE IV

Growth Response of Rats Fed Diets Containing
Various Carbohydrates

Exp. No.	Ration No.	Ration	Mean	Mean
			Weekly Gain in Wt. g.	Daily Food Intake g.
I	1.	potato starch	13.5	9.8
	2.	sucrose	21.9	9.7
	3.	autoclaved potato starch	22.2	9.9
	4.	glucose	22.2	9.7
	5.	dextrin	20.3	9.8
II	6.	potato starch	8.8	8.9
	7.	potato starch and lactose	17.5	9.6
	8.	sucrose	23.5	9.6
	9.	sucrose and lactose	22.1	9.6
III	10.	potato starch & sucrose	13.6	9.5
	11.	potato starch & sucrose and N	12.4	9.0
	12.	autoclaved corn starch	13.8	8.4
	13.	autoclaved corn starch and N	8.8	6.8
	14.	corn starch	10.8	8.2
	15.	corn starch and N	9.1	7.7

alteration in growth when 25% sucrose or lactose replaced an equivalent amount of corn starch in the basal ration. The beneficial effect obtained when unmodified potato starch was partially replaced by lactose may have been due to the utilization of lactose per se by the rat.

The rate of gain of rats was not improved by supplementing 9% casein rations with ammonium salts. Similar results were obtained by Lardy and Reldott (21) when they incorporated ammonium citrate into rations containing suboptimal amounts of fibrin or soybean oil meal.

Values presented in Table V indicate the effects of various carbohydrates on the progressive weekly excretion of lysine, valine, histidine, and methionine in the feces. Potato starch, autoclaved potato starch, and dextrin induced much greater excretion of amino acids in the feces than either sucrose or glucose. Mitchell, Hamilton and Beadles (28) found that sucrose, fructose, and lactose caused greater losses of organic nutrients in the feces than did glucose. They concluded that carbohydrates may have differential effects on the digestibility of the organic nutrients contained in the rations. In the study reported here, a significant loss of dietary protein was induced by potato starch, whereas dietary protein was almost completely digested and absorbed when either autoclaved potato starch or sucrose was used as the source of carbohydrate (Table IX).

TABLE V

Effect of Various Carbohydrates on the Weekly Levels
of Amino Acids in the Feces

No.	Ration	Wk. of Exp.	Lysine mg./wk.		Valine mg./wk.		Histidine mg./wk.		Methionine mg./wk.	
			M. [★] ± S.E. ^{★★}		M. ± S.E.		M. ± S.E.		M. ± S.E.	
1.	potato starch	1	48	9.0	50	3.3	18	2.1	17	1.7
		2	79	9.1	71	7.0	28	2.9	22	2.4
		3	98	6.2	101	9.8	37	3.0	33	2.0
		4	100	11.5	100	8.2	35	2.7	31	2.6
		5	112	10.5	108	9.6	35	2.5	39	2.2
2.	Sucrose	1	14	1.2	18	1.5	5.0	0.45	5.3	0.45
		2	14	1.0	17	1.0	5.1	0.71	5.5	0.39
		3	21	1.3	25	1.7	5.9	0.77	8.5	0.45
		4	23	1.8	27	1.6	6.3	0.74	9.1	0.77
		5	26	1.8	29	1.4	6.8	0.55	8.0	0.55
3.	auto- claved potato starch	1	48	3.3	52	3.0	13	0.9	14	0.7
		2	67	4.0	73	6.5	19	1.3	19	1.9
		3	71	6.0	83	6.1	21	1.2	24	1.5
		4	66	8.1	76	8.7	21	1.6	21	2.5
		5	80	9.3	81	9.1	30	2.8	24	2.1
4.	glucose	1	17	2.3	32	4.3	12	1.9	7.3	1.32
		2	14	3.4	26	4.2	10	1.4	6.3	1.10
		3	34	2.0	49	2.8	15	1.8	10.8	1.26
		4	20	2.6	27	2.9	9	1.1	6.6	0.92
		5	27	2.4	32	2.2	11	1.0	9.1	0.90
5.	dextrin	1	75	5.4	64	3.4	31	2.8	19	1.3
		2	73	13.9	60	11.6	30	6.4	18	2.6
		3	97	12.0	83	10.8	38	4.6	31	4.0
		4	75	10.2	73	9.9	31	4.0	24	3.4
		5	103	12.5	95	9.2	43	4.5	35	3.5

★ mean

★★ standard error of the mean

The extent to which dietary protein contributed amino acids in the feces was apparently modified by the type of carbohydrate fed.

The levels of amino acids excreted in the feces were lower or tended to be lower during the first week of the experiment than during the following weeks. During the first week the quantity of food consumed by the rats was least. Since Mitchell (27) has shown that the metabolic nitrogen excreted in the feces is proportional to the food intake, the smaller quantity of amino acids from endogenous sources could partly account for the lower levels of amino acids found in the feces during the first week.

That the intestinal secretions and the intestinal microorganisms probably contribute the major portion of the amino acids found in the feces is supported by the data in Table IX, which show that the metabolic nitrogen makes up a large portion of the nitrogen excreted in the feces. Also, unmodified potato starch and autoclaved potato starch, which induced relatively large excretions of metabolic nitrogen, were found to induce the highest levels of amino acids in the feces. It thus appears reasonable to conclude that the type of carbohydrate ingested by the rat influences the amounts of amino acids from endogenous sources which are found in the feces.

The effect of lactose on the fecal excretion of lysine,

TABLE VI

Effect of Lactose on the Levels of
Amino Acids in the Feces

<u>No.</u>	<u>Ration</u>	<u>Lysine</u> <u>mg./wk.</u>		<u>Valine</u> <u>mg./wk.</u>		<u>Histidine</u> <u>mg./wk.</u>	
		<u>M. $\star$ $\pm$ S.E. $\star\star$</u>		<u>M. $\pm$ S.E.</u>		<u>M. $\pm$ S.E.</u>	
6.	potato starch	67	6.2	88	7.0	38	2.4
7.	potato starch & lactose	73	4.9	88	3.9	41	2.9
8.	sucrose	21	0.9	26	1.3	7.7	0.22
9.	sucrose & lactose	29	2.1	31	2.4	9.0	0.45

$\star$ mean

$\star\star$ standard error of the mean

valine, and histidine is shown in Table VI. The incorporation of 20% of lactose into the potato starch ration apparently did not influence the amino acid content of the feces; however, the inclusion of 10% of lactose in the sucrose ration caused an increase in the fecal amino acid content. The t test was applied and the increase in the lysine and histidine values were found to be statistically significant ($t=3.73$, $P<0.01$; $t=2.80$, $P=0.02$). The increase in the valine value was not statistically significant ($t=1.64$, $P>0.1$). Ross (39,40) has shown that breast-fed infants excrete greater quantities of free amino acids in the feces than do infants receiving artificial diets. The high lactose content of human milk was mentioned as possibly influencing the amount of free amino acids excreted in the feces. Ross also found that in the feces of the breast-fed infant the predominant organism was Lactobacillus bifidus, which comprised almost the whole flora; whereas in the infant on artificial diets the fecal organisms were mixed, and L. bifidus was either scanty or absent. Mitchell (29) showed that there was an increase of Bacillus acidophilus in the intestinal flora of the rat when 15% of lactose was incorporated into the diet. It might be assumed then that the intestinal flora of the rats fed ration 8 (sucrose) was different from the intestinal flora of the rats fed ration 9 (sucrose & lactose). Possibly the higher levels of fecal amino acids obtained with ration 9,

TABLE VII

Effect of Ammonium Salts on the Levels
of Amino Acids in the Feces

<u>No.</u>	<u>Ration</u>	<u>Mean Lysine mg./wk.</u>	<u>Mean Valine mg./wk.</u>	<u>Mean Histidine mg./wk.</u>	<u>Mean Methionine mg./wk.</u>
10.	potato starch	82	80	38	18
11.	potato starch & N	91	88	42	22
12.	autoclaved c.s.	60	64	23	18
13.	autoclaved c.s. & N	56	57	21	16
14.	corn starch	25	28	10	6.8
15.	corn starch & N	18	21	9.5	5.0

TABLE VIII

Effect of Ammonium Salts on the Levels
of Amino Acids in the Feces

No.	Ration	Lysine/ Protein Intake <u>mg./g.</u>	Valine/ Protein Intake <u>mg./g.</u>	Histidine/ Protein Intake <u>mg./g.</u>	Methionin Protein Intake <u>mg./g.</u>
		<u>M. [★] ± S.E. ^{★★}</u>	<u>M. ± S.E.</u>	<u>M. ± S.E.</u>	<u>M. ± S.E.</u>
10.	potato starch	13.7 0.89	13.4 0.63	6.8 0.32	3.0 0.84
11.	potato starch & N	16.1 1.96	15.5 1.41	7.4 0.59	3.9 0.54
12.	autoclaved c.s.	11.8 0.97	12.2 0.67	4.3 0.22	3.5 0.32
13.	autoclaved c.s. & N	13.5 1.08	13.3 0.95	4.8 0.36	3.8 0.28
14.	corn starch	4.8 0.45	5.3 0.40	2.0 0.24	1.3 0.04
15.	corn starch & N	3.7 0.40	4.3 0.23	2.0 0.16	1.1 0.07

★ mean

★★ standard error of the mean

TABLE IX
Effect of Carbohydrates on the Digestibility
of Casein

<u>Ration</u>	<u>Rat No.</u>	<u>Initial Wt. g.</u>	<u>N Intake mg.</u>	<u>Fecal N mg.</u>	<u>Metabolic Fecal N mg.</u>	<u>True Digestibility %</u>
corn starch	1	80	80	11	9	98
	2	97	106	16	10	94
	3	71	114	12	11	99
potato starch	4	83	100	40	25	85
	5	84	126	49	29	84
	6	74	94	33	12	78
autoclaved potato starch	7	80	78	20	14	92
	8	92	101	24	19	95
	9	74	75	17	13	95
sucrose	10	69	56	6	5	98
	11	95	99	8	11	100
	12	66	81	4	6	100

as compared with ration 8, resulted from changes in the intestinal flora.

From Table VII, it can be seen that the average levels of the fecal amino acids determined for ration 10 are similar to the levels determined for ration 1 (Table V). Since ration 10 contained 9% casein, in contrast to ration 1 which contained 18% casein, there does not appear to be any correlation between the amount of protein in the diet and the amount of amino acids found in the feces.

The effect of autoclaved corn starch on the excretion of fecal amino acids was similar to the effect of the autoclaved potato starch. The levels of fecal amino acids induced by unmodified corn starch were approximately the same as the levels induced by sucrose. Corresponding results were obtained in the digestibility studies (Table IX). The amount of metabolic nitrogen excreted when unmodified corn starch was fed was approximately the same as that observed when sucrose was fed.

Since the food intake influences the amount of metabolic nitrogen excreted, Table VII does not offer a comparison between groups. The amounts of amino acids excreted per gram of protein ingested are reported in Table VIII. On this basis there was a tendency for an excretion of slightly higher levels of amino acids in the feces, with the incorporation of ammonium salts into rations containing either unmodified potato starch or autoclaved corn starch.

PART II

EFFECT OF CORN STARCH AND SUCROSE
ON THE GROWTH RESPONSE OF RATS

Experimental

Animal-feeding techniques

Groups of weanling rats of 45 to 60 gram body weight (22 to 26 days old) were fed experimental rations containing either corn starch or sucrose as the source of carbohydrate. Each group consisted of 3 males and 3 females except in experiment I.b, where 4 males and 2 females were used. The rats were housed in individual cages with raised screen bottoms and were given food and water ad libitum for a period of either 4 or 6 weeks. Each rat was weighed weekly and the food consumption for each was recorded every other day.

The basal rations used were the same as shown in Table I, with the exception that whole liver powder was omitted. The same levels of vitamins as shown in Table II were also used.

The experimental rations fed are shown in Table X. The supplements, reported as percentages of the ration were added at the expense of the carbohydrate.

TABLE X
Experimental Rations

Exp. No.	Level of Casein %	Ration
Ib	18	0.5% methionine and corn starch
	18	0.5% methionine and sucrose
	9	0.5% methionine and corn starch
	9	0.5% methionine and sucrose
II	9	0.5% methionine & sucrose and 0.4% liver concentrate★
	9	0.5% methionine & sucrose and double B vitamins★★
	9	0.5% methionine & corn starch and 0.4% liver concentrate★
III	9	0.5% methionine & sucrose and 1.05% of essential amino acids★★★
	9	0.5% methionine & corn starch and 1.05% of non-essential amino acids★★★★
	9	0.5% methionine & sucrose and 1.05% of non-essential amino acids★★★★
IV	9	0.5% methionine & sucrose and 1.0% sulfasuxidine
	9	0.5% methionine & corn starch and 1.0% sulfasuxidine

★ Wilson's liver concentrate 1:20.

★★ The B-vitamins and inositol were added to the ration at double the usual levels.

★★★ The following essential amino acids were added to the ration: 0.20% histidine, 0.40% lysine, 0.15% valine, 0.20% threonine, 0.10% tryptophan.

★★★★ The non-essential amino acids and the amounts added were as follows: 0.55% glutamic acid, 0.50% glycine.

Results and Discussion

The results of the growth experiments are shown in Table XI and Figure 1. The growth data for experiment I.a were obtained from experiments conducted in Part I and are presented here for comparison with the present results.

Rats, under restricted food intake, when fed rations containing 18% casein, grew well and at approximately the same rate with either corn starch or sucrose as the source of carbohydrate. The growth rate of the rats fed 9% casein with either corn starch or sucrose as the source of carbohydrate, was poor. Growth was undoubtedly limited by the suboptimal amounts of certain essential amino acids, in particular methionine, provided by the 9% casein diet.

When 0.5% methionine was included in the 18% casein diets, the growth rate was very good. Rats fed this diet with corn starch as the source of carbohydrate, grew at an average rate of 35 grams per week during the six weeks of the experiment. Rats fed a similar diet with sucrose as the carbohydrate, grew at an average rate of 33.3 grams per week.

In experiment I.b, the addition of 0.5% methionine to the 9% casein rations resulted in a growth rate of 24.0 grams per week when corn starch was used as the source of carbohydrate; whereas the growth rate when sucrose was used as the carbohydrate was only 16.3 grams per week. However, during preliminary experiments with equalized and

TABLE XI

Comparative Effect of Corn Starch and Sucrose
on the Growth Response by Rats

Exp. No.	Level of Casein %	Ration	Mean Gain in wt./wk. g.	Mean Daily Food Intake g.
I.a	18	corn starch (restricted)	21.8	9.6
	18	sucrose (restricted)	23.5	9.6
	9	corn starch (ad. lib.)	10.8	8.2
	9	sucrose (ad. lib.)	8.7	6.4
I.b	18	methionine & corn starch	35.0	14.0
	18	methionine & sucrose	33.3	13.2
	9	methionine & corn starch	24.0	12.2
	9	methionine & sucrose	16.3	9.1
II	18	methionine & sucrose	28.7	11.0
	9	methionine & sucrose	10.7	7.4
	9	methionine & corn starch	24.5	11.5
	9	methionine & sucrose and 0.4% liver	14.5	8.5
	9	methionine & sucrose and dbl. B-vitamins	15.0	8.5
	9	methionine & corn starch and 0.4% liver	23.2	11.1
	9			
III	18	methionine & sucrose	33.5	12.5
	9	methionine & sucrose	13.0	8.1
	9	methionine & corn starch	25.7	12.0
	9	methionine & sucrose and ess. amino acids	25.0	10.1
	9	methionine & sucrose and non-ess. amino acids	11.5	7.6
	9	methionine & corn starch and non-ess. amino acids	28.2	12.6
	9			
IV	9	methionine & sucrose	12.2	9.1
	9	methionine & corn starch	20.0	11.1
	9	methionine & sucrose and sulfasuxidine	13.5	8.9
	9	methionine & corn starch and sulfa suxidine	25.5	12.7

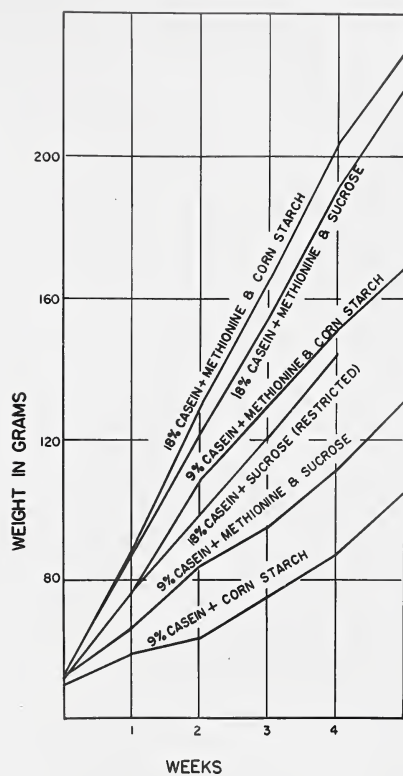


Figure 1. Growth curves for groups of rats fed rations containing either corn starch or sucrose as the source of carbohydrate.

restricted feeding, the growth rates of rats fed this ration were approximately the same regardless of whether corn starch or sucrose was used as the carbohydrate. Apparently, marked differences in growth between groups of rats fed corn starch and groups fed sucrose occurred only after the inclusion of 0.5% methionine in the rations and then only with ad libitum feeding.

The animals consumed less of the ration containing 9% casein, methionine, and sucrose than of the corresponding ration containing corn starch as the carbohydrate. The difference in food intake was not thought to be due to a difference in the palatability of the rations, because the food consumption was approximately the same for animals fed diets containing 18% casein and methionine, with either corn starch or sucrose as the carbohydrate. Further, with the inclusion of only 1% of a mixture of essential amino acids in the ration containing 9% casein, methionine and sucrose, the food consumption was greatly improved.

The differences in food intake were considered to be caused by differences in nutritional efficiency of the rations. Hegsted and Haffenreffer (12) found that for young rats the mean daily food intake varied as the mean body weight raised to the 0.88 power. They suggested that the food intake of young rats was controlled, by some means, at a relatively constant percent above the requirements for basal metabolism.

If the food consumed contained adequate nutrients for growth, the animal grew, its basal metabolism increased, and thus its food intake increased upon succeeding days. If the food eaten did not support normal growth, then the food intake was correspondingly decreased.

The results of experiment II show that an increase in the level of B-vitamins in the ration or the addition of liver concentrate did not substantially influence the growth rate of the rats fed the sucrose ration. The low mean gain in weight (10.7 grams per week) of the control group in this experiment can be accounted for by the failure of 2 rats to eat normally. Apparently, intestinal synthesis of vitamins, at least of the known vitamins and of any unknown growth factors contained in liver concentrate, does not explain the growth differences obtained between groups of animals fed corn starch and those fed sucrose rations.

Histidine, lysine, valine, threonine, and tryptophan were added to the 9% casein-sucrose ration in amounts equivalent to those used by Hall and Sydenstricker (8). The growth rate of the rats fed this ration was approximately the same as that of rats fed the 9% casein - corn starch ration. Apparently, the amino acid supplement overcame the factor which was limiting the growth of animals fed the sucrose ration. Hall and Sydenstricker suggested that corn starch promoted intestinal synthesis which made up small deficiencies of these essential

amino acids. If intestinal synthesis of essential amino acids occurred, it would appear that the dietary casein supplied sufficient nitrogen for this purpose, because the addition of non-essential amino acids to the corn starch ration did not cause an increase in the growth rate. Since marked differences were obtained between animals fed corn starch and those fed sucrose only when methionine was present in the ration, intestinal synthesis of this amino acid was not significant. Williams and Moir (47) suggested that the value of methionine as a supplement to urea in improving nitrogen retention in growing lambs was due largely to its stimulating effect on bacterial growth in the rumen, which increased the amount of bacterial protein available to the host. It is possible that methionine may play a similar role in the rat.

The addition of 1% sulfasuxidine did not appear to affect the growth rate of the rats fed either the 9% casein - sucrose or 9% casein-corn starch rations. Since the inclusion of sulfasuxidine did not cause a decrease in the rate of growth of the rats fed the corn starch diet, the intestinal bacteria which were inhibited by sulfasuxidine apparently did not play a role in the possible synthesis of essential amino acids.

Monson, Dietrich, and Elvehjem (30) found that the rate of growth of chicks was greater when dextrin rather than sucrose was used as the source of carbohydrate. Cellulose, sulfasuxidine, reticulogen, fish solubles, or vitamin B₁₂

did not change the significant growth difference observed when these carbohydrates were used. They suggested that dextrin favoured the synthesis of some unknown chick growth factor. However, in a preliminary investigation, Harper and Lepp (9) found that chicks fed a sucrose diet grew as well as those fed a dextrin diet when the level of protein in the sucrose diet was increased. These results are similar to the observations made during this investigation. Further study is required to explain the growth differences induced by various carbohydrates.

SUMMARY

The effect of various carbohydrates on the levels of amino acids excreted in the feces has been studied. Potato starch, autoclaved potato starch or dextrin each induced much greater levels of amino acids in the feces than either sucrose or glucose. Data from protein digestibility studies indicate that the slowly digested polysaccharides caused a greater excretion of metabolic nitrogen in the feces than either sucrose or glucose. Since the major portion of the fecal nitrogen consisted of metabolic nitrogen, the amino acids found in the feces were thought to be derived chiefly from endogenous sources. The digestibility studies also showed that potato starch induced losses of dietary protein in the feces.

The inclusion of 10% of lactose in the sucrose ration resulted in statistically significant increases in the levels of lysine and histidine in the feces. However, the incorporation of ammonium salts into 9% casein diets had no apparent effect on the amounts of amino acids in the feces.

A study was made to compare the growth of rats fed diets containing either corn starch or sucrose as the source of carbohydrate. Rats fed ad libitum a ration containing 9% casein and 0.5% methionine with corn starch as the source of carbohydrate, grew at a much higher rate than those fed a corresponding ration with sucrose as the source of carbohydrate. The addition of non-essential amino acids, B-

vitamins, a liver concentrate or sulfasuxidine did not change the marked differences observed. However, the nutritional efficiency of the sucrose ration was improved when histidine, valine, threonine, lysine, and tryptophan were included in small quantities. A possible explanation for the growth differences between animals fed corn starch and those fed sucrose was discussed.

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SECTION B

INADEQUACY OF UNMODIFIED POTATO STARCH AS DIETARY CARBOHYDRATE FOR THE ALBINO RAT

INTRODUCTION

It was noted during the preceding investigation that potato starch, in contrast to corn starch, was poorly utilized by the rat. However, when the potato starch was autoclaved or oven-baked it was rendered more digestible. This was shown by the increase in the growth rate of rats fed rations containing either of these modified starches as the source of carbohydrate. Further study of potato starch as a dietary carbohydrate seemed warranted by these observations.

A review of the literature revealed scattered reports published over the past half century which gave clear evidence that potato starch was poorly utilized. Several investigators (5,9,10,1) found that the human body manifested much greater efficiency in the digestion of starches from cereal grains than in the digestion of starch from potatoes. Raw potato starch has also been shown to be poorly utilized by rats (8), dogs (4), guinea pigs (12), hens (7), mice (3), and sheep (6). The digestibility of potato starch was shown to be greatly improved by cooking or by mechanically grinding the starch (11).

The present investigation was designed to study further the factors influencing the utilization of raw potato starch. The methods used in the pursuit of this objective were:

(a) alteration of the rations in an attempt to improve the

utilization of potato starch by the rat; (b) a comparison of wheat, corn, and potato starches which were prepared in the laboratory without the use of chemicals; (c) a study of enzyme digestion of starches in vitro.

Experimental

Animal-feeding techniques

Groups of weanling male rats of 40 to 50 grams body weight (21 to 25 days old) were used as the experimental animals. The 5 rats which comprised each group were housed in individual cages with raised screen floors and were given water ad libitum. As in Section A, Part I, the food intake was controlled by restricting the amount of ration offered each rat to 10 grams per day. Each animal was weighed weekly and individual food consumption records were kept.

In experiment I, rats were fed 18% casein rations (Tables I and II), containing unmodified commercial potato starch as the source of carbohydrate, for a period of 4 weeks. Each ration contained one of the following supplements which was incorporated at the expense of potato starch: 7.5% cellufLOUR; 5% lactose; 20% lactose; 5% sucrose; 20% sucrose. To maintain equivalent caloric intakes, the rats fed the ration which was supplemented with 7.5% cellufLOUR were each offered 10.7 to 10.8 grams of ration daily. The utilization of the carbohydrate component was assessed by (a) its capacity to promote growth and (b) its effect on the quantity of feces excreted by the rat.

In experiment II, laboratory preparations of corn, wheat, and potato starches were compared as the source of carbohydrate in 20% casein rations. The composition of these rations was

basically the same as shown in Tables I and II, but 20% casein was substituted for 18% casein at the expense of the carbohydrate. After the experimental period of 3 weeks, one rat from each group was sacrificed and the gastrointestinal tracts were compared.

Preparation of starches

Wheat starch was prepared by working wheat flour into a paste with a small amount of water and extracting the paste several times with water to separate starch from the gluten. The starch granules were allowed to settle from the washings and the supernatant liquid was decanted. After repeated washings with water, the starch was air-dried at approximately 60°C.

Potato starch was prepared from potatoes which were peeled, washed, and put through a meat grinder. The minced product was mixed thoroughly with water and then the pulp was separated from the starch granules by filtering the mixture through cotton cloth. After allowing the starch granules to settle, the supernatant liquid was decanted. After repeated washings with water, the starch was air-dried at approximately 60°C.

Corn starch was prepared by shaking a quantity of corn meal with about 5 volumes of water in a large glass jar for one hour on the mechanical shaker. The starch granules, suspended in water, were transferred to another container, and

allowed to settle. The supernatant liquid was discarded and the corn starch thus obtained was air-dried at approximately 60°C.

Ground potato starch was prepared by pulverizing commercial potato starch for 4 hours in a ball mill containing porcelain balls.

Enzyme digestion studies

The rate of hydrolysis by pancreatin was determined in vitro for each of the starches prepared in the laboratory. The enzyme solution was prepared by shaking 250 mg. of pancreatin with 50 ml. of distilled water. The solution was centrifuged and the supernatant liquid was used as the source of alpha-amylase.

The rate of hydrolysis of each of the starches was determined as follows: 250 mg. of the starch was placed in a 50 ml. rubber-stoppered cylinder. 5 ml. of the enzyme solution, 2 ml. of phosphate buffer[★] (pH 7), and distilled water to provide a total volume of 50 ml. were added. The starch granules were suspended in the liquid by shaking and then the cylinder was placed in an incubator at 30°C. 5 ml. samples were taken from the cylinder after 0, 1, 3, and 20 hours of incubation and the reducing power, expressed as maltose, was determined by the Somogyi method (14).

★ M/15

Results and Discussion

The growth response of rats fed the supplemented potato starch rations and the respective average weights of the feces are shown in Table XII. The incorporation of 7.5% celluflour into the basal ration did not appear to influence the utilization of potato starch by the rat. The increase in the weight of the feces excreted by rats fed this ration was probably due to the presence of undigested 'celluflour.' The inclusion of 5% of lactose or sucrose in the basal ration resulted in a slight increase in the rate of growth with a coincident decrease in the amount of feces excreted. The incorporation of 20% of lactose or sucrose into the basal ration accentuated the increase in the rate of growth and the decrease in the amount of feces. It seems probable that the dissacharides were completely or almost completely assimilated, while the potato starch in the ration remained poorly utilized.

The data for experiment I.b were obtained from experiments of Section A, Part I, and are presented for comparison with the present results. The growth rate of rats fed rations containing autoclaved potato starch or dextrin as the source of carbohydrate was approximately the same as that of rats fed rations with sucrose as the carbohydrate. Apparently, potato starch which was autoclaved or baked was well utilized by the rat.

TABLE XII

Effect of Various Starches on the Growth Response
of Rats

<u>Exp.</u> <u>No.</u>	<u>Ration</u>	Mean	Mean
		Gain in wt/wk. g.	Weekly wt. of Air-dried feces g.
I	potato starch	13.5	28.7
	potato starch & 7.5% cellufLOUR	14.7	33.4
	potato starch & 5% lactose	16.8	25.5
	potato starch & 20% lactose	19.1	22.6
	potato starch & 5% sucrose	16.8	25.4
	potato starch & 20% sucrose	19.4	21.8
	sucrose (control)	22.6	2.1
I.b	autoclaved potato starch	22.2	4.8
	dextrin	22.2	9.3
	sucrose (control)	21.9	1.7
II	potato starch (lab. prep.)	16.5	
	wheat starch (lab. prep.)	28.6	
	corn starch (lab. prep.)	29.8	
	sucrose (control)	32.3	

In experiment II, the laboratory preparations of corn starch and wheat starch each supported a rate of growth which was only slightly lower than that induced by sucrose. Two rats, when fed the 20% casein ration with ground potato starch as the source of carbohydrate, grew at an average rate of 23.0 grams per week. It would appear that wheat starch and corn starch are well utilized, and that grinding of the potato starch improves its utilization by the rat. Langworthy and Deuel (9) found that raw corn and wheat starches were completely utilized by human subjects.

Meteorism (an extension of the abdomen by gas), which occurs with the ingestion of unmodified potato starch, was exhibited by rats fed the laboratory preparation of potato starch. A comparison of the gastro-intestinal tracts of the rats fed the different starches is shown in Figure 2. The ingestion of unmodified potato starch caused a distension of the caecum and intestines. The main causative factors for this enlargement was thought to be due to the accumulation of food residue in the caecum and the formation of gases by fermentation of the food residue. In a previous experiment, it was found that the average dry weight of the gastrointestinal tracts of 5 rats fed an 18% casein ration with potato starch as the source of carbohydrate was 9.9 grams, whereas the corresponding average weight when rats were fed a ration with sucrose as the carbohydrate was 3.5 grams.

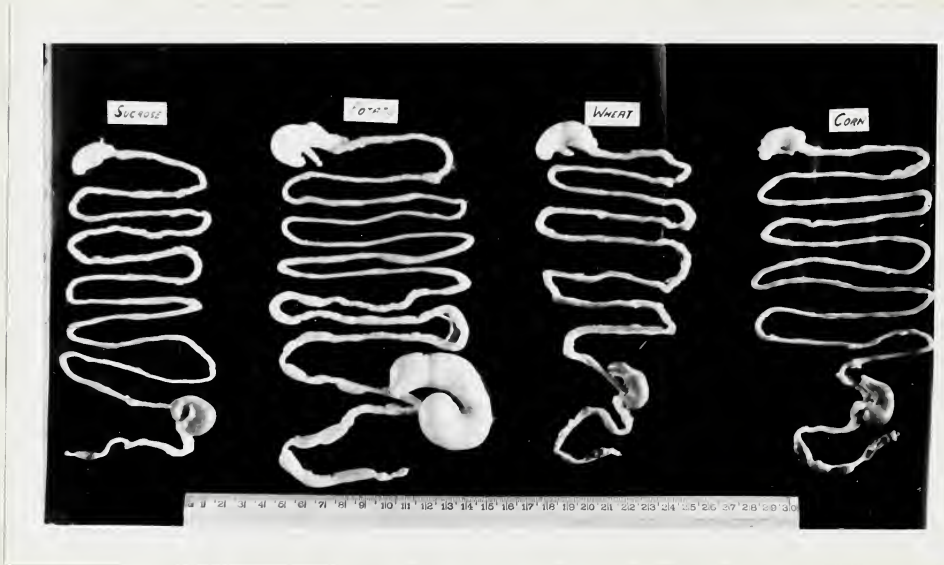


Figure 2. The gastrointestinal tracts of rats fed rations containing different carbohydrates.

This marked difference in the weights of the gastrointestinal tracts was probably caused by a greater accumulation of food residue in the caecum, when potato starch was ingested. Langworthy and Deuel, working with human subjects, found that the ingestion of potato starch caused excessive formation of gas and frequent intestinal cramps. Beazell, Schmidt, and Ivy (1) later confirmed these findings and further suggested that bacterial fermentation may account for an important part of the breakdown of unmodified potato starch which occurred during passage through the alimentary tract.

The extent of hydrolysis by pancreatin, of ground potato starch, wheat starch, corn starch, and unmodified potato starch is illustrated in Figure 3. Ground potato starch was hydrolyzed very rapidly whereas the hydrolysis of unmodified potato starch was very slight. Pronounced hydrolysis occurred with both corn starch and wheat starch, but probably the presence of fatty or waxy material in the crudely prepared starches interfered with the action of amylase. Considerable growth of microorganisms was noticed in the corn starch suspension after 20 hours of incubation and since reducing sugars had been consumed, the extent of hydrolysis of the corn starch could not be determined at this point. Sherman, Walker, and Caldwell (13) found that boiled potato starch showed a rate of enzymic hydrolysis equal to or slightly greater than that of the

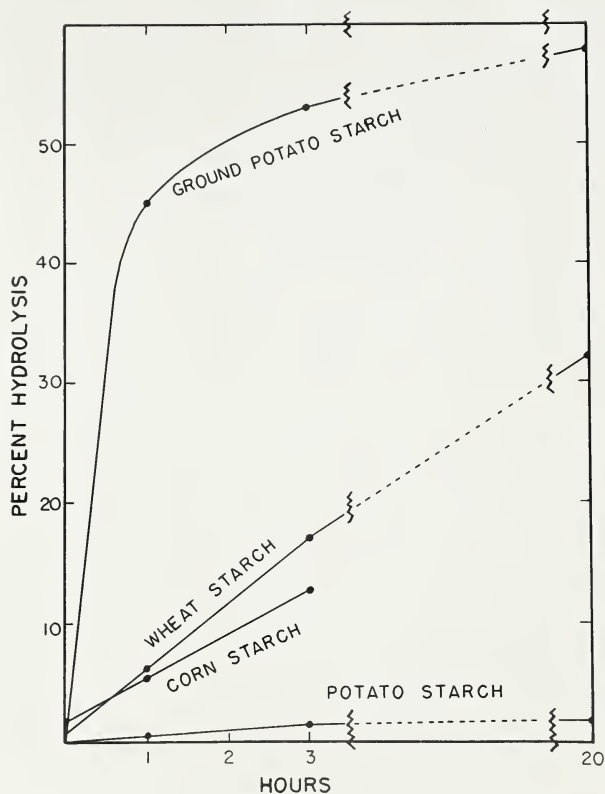


Figure 3. Rate of hydrolysis of starches by alpha-amylase (pancreatin). The reducing sugar was calculated as maltose.

cereal starches. Beazell et al. found that the quantity of reducing sugar obtained from raw potato starch never exceeded 7.6% of that obtained from the digestion of boiled starch. Since lengthening the incubation period to 20 hours did not increase the quantity of raw potato starch digested, they suggested that as a result of mechanical rupture of the envelope of the starch granule during milling, a constant fraction of the raw starch may have been more easily digested than the remainder. In view of the results obtained by these investigators and the observations made during this study, it is likely that unmodified potato starch is appreciably digested by alpha-amylase only when the outermost layer or layers of the starch granules are ruptured.

The observations made during this investigation were recently confirmed by Booher, Behan, and McMeans (2) in a report on the biological utilizations of unmodified and modified food starches. They suggested that the relatively low digestibilities of such unmodified starches as those from the potato, sago palm, and arrowroot are entirely attributable to a firmer degree of hydrogen-bonding of the molecules of the outermost layer or layers of the granules than occurs with starch granules from the cereal grains and cassava root.

As a matter of general interest, it was noted that the "true" digestibility of the dietary protein appeared to

vary directly with the digestibility of the carbohydrate component. The data from the protein digestibility study (Table IX) shows that the dietary protein was almost completely digested when either autoclaved potato starch or corn starch was used as the source of carbohydrate; whereas the digestibility of dietary protein was decreased when potato starch was the source of carbohydrate.

SUMMARY

Unmodified potato starch was poorly utilized, whereas corn starch and wheat starch were almost completely digested by the rat. The ingestion of unmodified potato starch caused a distension of the caecum and intestines. This enlargement resulted from the accumulation of food residue in the caecum and the consequent formation of gas from fermentation. The utilization of unmodified potato starch did not appear to be improved by the incorporation of either lactose or sucrose into the ration. However, autoclaving, baking or grinding improved the utilization of potato starch by the rat.

Digestion studies performed in vitro with pancreatin showed that pronounced hydrolysis of either corn starch or wheat starch occurred, but the hydrolysis of unmodified potato starch was very slight. Ground potato starch, however, was hydrolyzed very rapidly. The low digestibility of unmodified potato starch was attributed to the resistance of the outermost layer or layers of the starch granules to attack by alpha-amylase.

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